Journal of Apicultural Research

ISSN: 0021-8839 (Print) 2078-6913 (Online) Journal homepage: www.tandfonline.com/journals/tjar20

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To cite this article: Dominik Klier, Pia Spiegel & Johannes Novak (2025) The composition of volatile compounds of Alpine Rhododendron honey, Journal of Apicultural Research, 64:5, 1343-1351, DOI: 10.1080/00218839.2025.2543100

To link to this article: https://doi.org/10.1080/00218839.2025.2543100

Published online: 19 Aug 2025.

JOURNAL OF APICULTURAL RESEARCH
2025, VOL. 64, NO. 5, 1343–1351
https://doi.org/10.1080/00218839.2025.2543100

ORIGINAL RESEARCH ARTICLE

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The composition of volatile compounds of Alpine Rhododendron honey

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ABSTRACT

Rhododendron honey, also known as "Almrauschhonig," is a type of honey sourced from the nectar of *Rhododendron ferrugineum*, *Rhododendron hirsutum*, and their hybrid *R.* x *intermedium* found in the Alpine region. Volatile compounds were analysed to verify Rhododendron honey, using samples from the Alpine region, including "flower honey," "honeydew honey," "flower/honeydew honey mix," "mountain honey," and "Rhododendron honey." Before evaluating the volatile compounds, four physicochemical standard criteria indicated a mislabelled sample. However, none of the four traits allowed for the differentiation of Rhododendron honeys from floral honeys. Rhododendron honeys exhibited distinct differences from all other types in eight specific volatile compounds (limonene/β-phellandrene, linalool and lilac aldehyde stereoisomers A and B, *p*-cymene, pinocarvone, *trans*-3-caren-2-ol, and the fruity ketone 1,4-dimethyl-δ-3-tetrahydroacetophenone). On the other hand, honeydew honeys could be distinguished by two compounds, dehydrosabinene and *cis*-linalool oxide. Linear discriminant functions were computed for the three categories: flower, honeydew and Rhododendron honey. Compounds that were significant between the three groups were normalised and used in calculations. Because of this constraint, the model achieved a 90% accuracy rate. Out of the nine Rhododendron samples, seven were confirmed to be Rhododendron honeys using cross-validation. One of the mislabelled samples was a pure flower honey, while the other one was a mix of honeydew honey and Rhododendron honey. In summary, three samples of honeys that were not labelled as Rhododendron honeys were found to be Rhododendron honeys. This suggests that beekeepers missed the chance to market them as a unique and desirable honey variety.

ARTICLE HISTORY
Received 17 June 2024
Accepted 17 May 2025

KEYWORDS
Rhododendron ferrugineum L; *Rhododendron hirsutum* L; honey; discriminant analysis; volatile compounds; solid phase microextraction; gas chromatography

Introduction

Monofloral Rhododendron honey, also known as "Almrauschhonig" in German, is a unique type of honey that comes from alpenroses (*Rhododendron hirsutum*, *Rhododendron ferrugineum* and their hybrid *R.* x *intermedium*) found at high altitudes in the mountains at mountainous to subalpine levels (Fischer et al., 2008). Being a speciality, it is susceptible to food fraud or errors. Beekeepers may mistakenly categorise Rhododendron honeys as honeydew, flower, or mountain honeys, either due to a lack of awareness of their true worth or uncertainty regarding their classification. Rhododendron honey is characterised by an underrepresentation of *Rhododendron* pollen (30–60%) and by its very light colour, low conductivity and ash levels (Persano Oddo et al., 1995).

The classic melissopalynology (Ohe et al., 2004) is considered the gold standard for specifying honeys. However, analysis of physicochemical parameters conducted in the routine assessment of honey is the most common method for detecting botanical sources (Juan-Borrás et al., 2014). Water content, pH, free acidity and electrical conductivity are some of the standard physicochemical parameters proposed by the European Honey Commission, of which electrical conductivity is regarded as a good criterion of botanical origin (Bogdanov, 1997; Persano Oddo et al., 1995). Electrical conductivity mostly depends on its mineral content and is different between diverse botanical origins of honeys (Bogdanov, 1997; Pascual-Maté et al., 2018; Persano Oddo & Piro, 2004). Flower honeys usually have lower limits than honeydew honeys (Bogdanov, 1997). Water content and pH values are first-hand stability parameters, but the pH also indicates botanical origin (group), with honeydew honeys showing lower pH than flower honeys (Bogdanov, 1997). Free acidity is correlated to organic acids in equilibrium with their corresponding lactones or internal esters, and some inorganic ions, such as phosphate (Finola et al., 2007) and may be attributed to botanical sources or to the harvest season (Pérez-Arquillué et al., 1995).

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The volatile organic chemicals found in honey contribute to its sensorial qualities, such as aroma, and they are useful for determining its origin by general profiles as fingerprints or some individual components as markers (Pascual-Maté et al., 2018). The isolation of volatile compounds is not trivial, and different methods have been developed for this purpose (Machado et al., 2020). Alissandrakis et al. (2005) conducted a comparative analysis of four distinct procedures for isolating honey volatile compounds: hydrodistillation (HD), micro-simultaneous steam distillation—solvent extraction (MSDE), ultrasound-assisted extraction (USE), and solid-phase microextraction (SPME). While HD and MSDE involve sample heating, which can result in the formation of artefacts, USE and SPME did not alter the inherent composition of honey volatile compounds. Solid-phase microextraction (SPME) is a solvent-free extraction method using a glass fibre coated with an adsorbent for quick sample preparation (Boyacı et al., 2015; Kudlejova & Risticevic, 2012; Pawliszyn, 1997). SPME is the most common method used to isolate volatile compounds from honey (Jerković & Marijanović, 2009). Isolation from the gas phase above the sample is followed by separation, identification, and quantification of volatile compounds with gas chromatography coupled to mass spectrometry (GC/MS). SPME is a solvent-free, fast and straightforward sample isolation and preconcentration technique, fulfilling most of the criteria of *green analytical chemistry*. Sampling the headspace also reduces sample matrix effects by unspecific adsorption on the fibre (Lancioni et al., 2022). Transitions of volatiles from sample to gas phase, adsorption to fibre are complex, demanding careful method optimisation.

This study aimed to compare the volatile profiles of various types of commercial honeys from Alpine regions, including Rhododendron honey, flower honey, honeydew honey, flower/honeydew honey and mountain honey.

Materials and methods

Honey samples

In the spring of 2023, a total of 49 honey samples were acquired *via* beekeepers, retail stores, or online sources from Alpine regions in Austria. The vendors labelled the honeys as "Almrauschhonig" (Rhododendron honey, monofloral, $n = 10$), "Blütenhonig" (flower honey, multifloral, $n = 13$), "Gebirgshonig" (mountain honey, multifloral, $n = 8$), "Waldhonig" (honeydew honey, "multifloral," $n = 11$) or "Wald-/Blütenhonig" (a mix of flower and honeydew honey, multifloral, $n = 7$). The honeys were stored in the dark at room temperature until analysis.

Water content, pH, free acidity and electrical conductivity

Moisture of honeys was measured using a HHTEC RHB-90 honey refractometer (Hong Han). Conductivity, pH and free acidity were determined according to the harmonized methods of the European Honey Commission (Bogdanov, 1997). In brief, electrical conductivity was examined by dissolving 20 g of honey, based on dry weight, in 100 mL of MilliQ® water. The measurement was conducted using an Ino Lab pH/Cond multiparameter measuring instrument (WTW). The determination of free acidity and pH was performed by titration. 10 g of honey sample (fresh weight) was dissolved in 75 mL MilliQ® water and agitated using a magnetic stirrer. The pH electrode was immersed, pH was measured, and the solution was titrated using 0.1 M NaOH until the pH reached 8.3. The measurement of free acidity in honey was quantified as milliequivalents of acid/kg of honey. All physicochemical parameters were analysed in duplicate.

SPME-GC/MS

Volatile organic compounds (VOCs) were transferred into the GC by adsorption of the volatile compounds on a PDMS/DVB SPME-fibre (Supelco® SPME Fibre Assembly, 65 µm PDMS/DVB, Merck) and desorption in the injector of the GC/MS. The weight of the samples, as well as adsorption temperature and duration, were optimized based on an in-house method (data not shown). 1 g of the honey sample was weighed into a 20 mL glass vial. 1 mL MilliQ® water was added. The vial was then closed with a PTFE-coated butyl septum (VWR International) and placed in the autosampler of the GC/MS (CombiPAL, CTC Analytics). The samples were incubated for 20 min at 50 °C under regular shaking (2 s at 250 rpm, 10 s intermission) to reach phase equilibrium. Following the process of equilibration, the fibre was exposed for 30 min under identical conditions. Subsequently, the fibre was passed into the GC, where the VOCs that had been adsorbed were desorbed in the GC injector at 250 °C (split ratio 1:1). The fibre was left in the GC injector for 2 min to guarantee complete desorption.

GC-MS analysis was performed on an Agilent 7890 A coupled to a 5957 C VL MSD (Agilent Technologies). The separation was performed on an HP-5MS column (30 m × 250 µm × 0.25 µm, Agilent Technologies) using a constant flow rate of 2 mL/min. He was used as the carrier gas. The initial oven temperature was set at 50 °C for a duration of 5 min. It was then raised to 140 °C at a rate of 3 °C/min, and followed by an increase of 20 °C/min until reaching a final temperature of 280 °C.

The chromatograms were examined using MassHunter Version 10.2 (Agilent Technologies). The compounds were identified by comparing their mass spectra with the NIST/EPA/NIH EI Mass Spectral Library (NIST 20) using the Probability Based Matching (PBM) search program as implemented in MassHunter 10.2. In addition, linear retention indices, calculated based on retention times of a homologous series of n-alkanes C9-C29 (Merck), were compared with values described in the literature. Data are presented in percentages, normalized to the total peak area of each sample.

Statistical analysis

The statistical analysis was conducted using R 4.2.3 (R Core Team, 2023) within the RStudio 2023.09.0 environment (Posit Team, 2025). The physicochemical properties and volatile compounds of different varieties of honey were evaluated using ANOVA, followed by a multiple comparison test of significant compounds with Bonferroni correction. A linear discriminant analysis was conducted utilising Rhododendron, flower and honeydew honeys and the relevant significant compounds between the three groups (octane, dehydrosabinene, dimethyltrisulfide, *p*-mentha-1,5,8, triene, *p*-cymene, limonene/β-phellandrene, phenylacetaldehyde, *cis*-linalool oxide, linalool, hotrienol, lilac aldehyde A, 1,4-dimethyl-δ-3-tetrahydroacetophenone, lilac aldehyde B, pinocarvone and an unknown compound with retention index 1163) using packages MASS (Venables & Ripley, 2007) and caret (Kuhn, 2008). The significant compounds were normalised before calculations.

Results

Four physicochemical parameters

At the beginning, four physicochemical parameters (water content, pH, free acidity and electrical conductivity) were analysed. The water content of the different types of honey did not vary ($F = 0.351$, $p = 0.842$) and was approximately equal to the overall mean water content of 15.9 g/100g (Table 1).

The honey types differed significantly in pH ($F = 24.52$, $p = 1.02^{-10}$), free acidity ($F = 3.435$, $p = 0.0158$) and electrical conductivity ($F = 15.17$, $p = 7.22^{-8}$).

The pH levels of Rhododendron honeys and flower honeys were identical, with a value of 4.1. This was then followed by mountain honeys and honeydew/flower honeys. Honeydew honeys exhibited the highest pH value of 4.9. The free acidity ranged from 21.9 mEq/kg (flower honeys) to 31.6 mEq/kg (honeydew honeys). Rhododendron honeys exhibited significant differences only when compared to honeydew honeys. The measure that provided the most accurate distinction among the samples was electrical conductivity. The electrical conductivity of honeydew honeys, measured at 1,055 µS/cm, was considerably different from all other forms of honey. The group of mountain honeys and honeydew/flower honeys had an average electrical conductivity of approximately 730 µS/cm. The combination of Rhododendron honeys and flower honeys created a cluster with a relatively low electrical conductivity of approximately 400 µS/cm. Based on the analysis of four physicochemical properties, it was not possible to differentiate Rhododendron honeys from floral honeys. Three honey samples were excluded from further examination because they, obviously, fell outside the typification boundaries.

Composition of volatile compounds

A total of 34 out of the 36 volatile compounds were identified (Table 2). When performing ANOVA on a compound-by-compound basis, only Rhododendron honeys and honeydew honeys exhibited differences in some compounds. The multifloral flower and mountain honeys exhibited such a high degree of heterogeneity that they could not be differentiated.

Rhododendron honeys exhibited a profile of volatile compounds distinct from all other types analysed, with eight significantly different components, most of them terpenoids. These substances include limonene/β-phellandrene, linalool, lilac aldehyde stereoisomers A and B, *p*-cymene, pinocarvone, *trans*-3-caren-2-ol, and the fruity ketone 1,4-dimethyl-δ-3-tetrahydroacetophenone.

Honeydew honeys differed from other forms of honey in two compounds, namely dehydrosabinene (syn. thuja-2,4(10)-diene) and *cis*-linalool oxide. The concentration of dehydrosabinene was found to be 4.2%, whereas in other forms of honey it ranged from 0.9% (Rhododendron honey) to 2.6% (mountain honey).

Table 1. Water content, pH, free acidity and electrical conductivity of different Austrian honeys.

Honey types	*n*	Water content [g/100g]	pH	Free acidity [mEq/kg]	Electrical conductivity [µS/cm]
Rhododendron honeys	10	16.0 *a*	4.1 *a*	23.3 *bc*	381 a
Flower honeys	13	15.9 *a*	4.2 *a*	21.9 *c*	409 a
Mountain honeys	8	16.1 *a*	4.5 *b*	28.6 *ab*	748 b
Honeydew/flower honeys	7	15.8 *a*	4.5 *b*	27.8 *abc*	717 b
Honeydew honeys	11	15.7 a	4.9 *c*	31.6 *a*	1,055 c

Honey types with the same letter do not differ significantly from each other in the respective parameter.

Table 2. Composition of volatile compounds of different honey types.

Compound	RI	Rhododendron honey		Flower honey		Honeydew honey		Flower/ Honeydew		Mountain honey		p	sig.level[b]
2-Methyl-butyronitrile	719	0.28[a]		0.28		0.87		1.62		1.61		0.204	
3-Methyl-butyronitrile	725	0.69		0.86		1.66		6.20		6.24		0.052	
3-Penten-1-ol	728	0.16		0.18		0.09		0.22		0.13		0.612	
Dimethyldisulfide	738	0.24		3.13		0.40		0.54		1.10		0.265	
Toluol	760	0.78		1.14		0.37		1.39		1.56		0.698	
1-Octene	788	0.97		0.37		1.11		0.48		0.80		0.140	
Octane	800	9.98	a	5.43	a	16.00	a	4.49	a	13.36	a	0.049	*
2,4-Dimethyl-2,3-heptadien-5-yne	829	0.40		0.39		0.16		0.37		0.28		0.752	
Dehydrosabinene	949	0.87	a	1.60	a	4.17	b	2.15	ab	2.59	ab	1.2^{-4}	
Benzaldehyde	958	8.20		11.66		17.77		17.51		7.85		0.128	
Dimethyltrisulfide	963	0.09	a	1.41	b	0.17	a	0.30	ab	0.34	ab	0.028	*
2-Pentylfuran	991	1.29		2.09		2.56		0.84		0.88		0.123	
α-Phellandrene	1001	0.70		0.82		1.01		0.71		0.76		0.541	
p-Mentha-1,5,8-triene	1002	0.81	a	1.75	b	1.90	b	1.44	ab	1.27	ab	0.029	*
Unknown	1005	3.10		2.60		2.54		2.48		3.54		0.660	
α-Terpinene	1013	1.58		1.30		2.21		1.26		1.81		0.402	
3-Ethenyl-1,2-dimethyl-1,4-cyclohexadiene	1019	2.74		2.96		2.24		2.09		2.02		0.054	
p-Cymene	1021	11.98	b	5.66	a	7.61	a	4.85	a	7.79	a	1.55^{-4}	***
Limonene/β-phellandrene	1025	2.37	b	1.05	ab	1.04	a	0.80	a	1.17	ab	0.009	**
Phenylacetaldehyde	1042	0.11	a	0.64	ab	2.14	b	2.52	b	1.10	ab	0.008	**
γ-Terpinene	1055	3.44		2.61		4.40		2.64		3.96		0.248	
cis-Linalool oxide	1070	4.13	c	3.15	bc	0.91	a	1.77	ab	1.46	ab	2.93^{-4}	***
p-Cymenene	1085	13.06		20.21		16.16		18.73		16.26		0.801	
Linalool	1097	7.21	b	1.55	a	2.06	a	1.46	a	3.39	ab	4.82^{-4}	***
Hotrienol	1103	6.48	ab	17.99	b	1.35	a	14.50	ab	8.30	ab	0.003	**
cis-Rose oxide	1107	0.34		0.29		0.31		0.24		0.38		0.768	
trans-3-Caren-2-ol	1135	2.13	a	0.55	a	0.46	a	0.49	a	0.50	a	0.047	*
Lilac aldehyde A	1139	1.27	b	0.44	a	0.16	a	0.35	a	0.77	ab	5.87^{-4}	***
1,4-Dimethyl-δ-3-tetrahydroacetophenone	1144	1.93	b	0.35	ab	0.52	a	0.32	a	0.51	a	0.013	*
Lilac aldehyde B	1147	2.49	b	0.74	a	0.29	a	0.63	a	1.43	ab	4.66^{-4}	***
Nerol oxide	1151	0.34		0.58		0.20		0.36		0.26		0.139	
Pinocarvone	1155	2.13	b	0.29	a	0.50	a	0.18	a	0.44	a	5.39^{-6}	***
Unknown	1163	0.57	a	0.28	a	0.17	a	0.29	a	0.46	a	0.075	
Terpinen-4-ol	1171	0.49		0.27		0.55		0.26		0.32		0.079	
p-Cymen-8-ol	1182	0.17		0.50		0.43		0.57		0.26		0.110	
β-Damascenone	1377	0.82		0.70		0.54		0.93		0.93		0.198	

[a]Values are mean values of percentages of the respective compound to the total peak area of each sample. [b]Levels of significance. $*p < 0.05$. $**p < 0.01$. $***p < 0.001$. Honey types with the same letter within each compound are not statistically different.

Authentication of rhododendron honey by discriminant analysis

Out of the five kinds of honey that were tested, three of them provided clear information on the origins of the honey flow. However, it should be noted that two of these types (flower honeys and honeydew honeys) have a wide range of sources for their honey flow. The name "Mountain honey" denotes honey sourced from elevated terrains (a topographical classification, not a honey flow designation). On the other hand, "honeydew/flower honey" denotes a blend of honey derived from both flowers and honeydew. This is the reason the discriminant functions were finally calculated only with Rhododendron, flower and honeydew honeys. In addition, only the compounds that showed significant differences among the three groups (octane, dehydrosabinene, dimethyltrisulfide, *p*-mentha-1,5,8, triene, *p*-cymene, limonene/β-phellandrene, phenylacetaldehyde, *cis*-linalool oxide, linalool, hotrienol, lilac aldehyde A, 1,4-dimethyl-δ-3-tetrahydroacetophenone, lilac aldehyde B, pinocarvone and an unknown compound with retention index 1163) were included in the analysis.

The initial linear discriminant analysis (LDA), including all five honey groups, yielded an accuracy (Acc) of 0.63 and a baseline, termed the "no information rate" (NIR), of 0.239 (p-value (Acc > NIR) = 3.217–10) (Figure 1A). Excluding "mountain honey" and "flower/honeydew" yielded a model with a high accuracy of 0.903, whereas NIR exhibited an accuracy of 0.35 (*p*-value (Acc > NIR) = 3.22–10) (Figure 1B), indicating robust discriminatory capability. The floral and honeydew honey categories, albeit encompassing a diverse array of honey flow sources, may be distinctly identified from one another with a high degree of precision. LDA computed two linear discriminant functions of nearly similar significance, with the Eigenvalues to the entire sum being LDA1 = 0.527 and LDA2 = 0.473.

The samples from all three groups were classified using a cross-validated approach known as "leave one out" (Table 3). The reclassification of Rhododendron honey was accurate for seven out of the nine samples. Out of the two incorrectly categorised Rhododendron labelled samples, one was solely flower honey, while the other was a combination of Rhododendron (with a 40% posterior probability) and honeydew honey (with a 60% posterior probability). One of the samples consisted of a combination of Rhododendron and honeydew, with a posterior

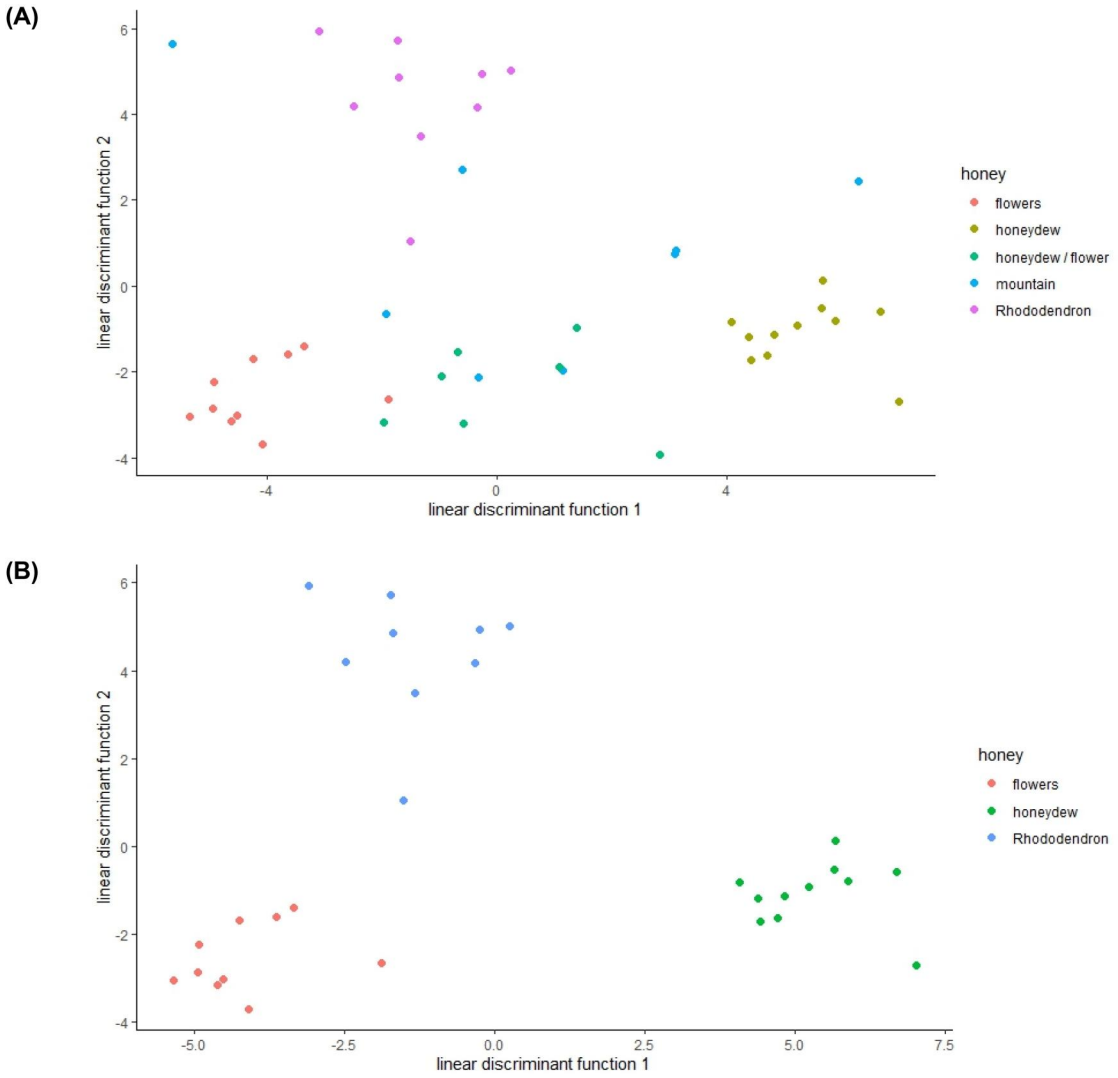

Figure 1. Scatter plot of the two discriminant functions, including all five (A), only three (B) honey classes for calculating the model (explained variances of linear discriminant function 1 are 53% for both variants and explained variances of function 2 are 41% and 47%, respectively).

probability of 43% Rhododendron and 57% honeydew. In contrast, all other Rhododendron samples had posterior probabilities of 100% Rhododendron.

Out of ten floral honeys, nine were accurately categorised, while one was identified as Rhododendron honey with a 94% posterior probability. All eleven honeydew honeys were accurately categorised. The mountain honeys consisted of either pure flower honey, pure honeydew honey or mixtures of both. Two samples were pure Rhododendron honeys. Out of the seven samples labelled as flower/honeydew mixes, three were mostly flower honeys, three mostly honeydew honeys and just one was a mixture of 25% flower and 75% honeydew (percentages are posterior probabilities).

Discussion

The objective of the study was to determine whether there are variations in the volatile compounds of Rhododendron honey compared to floral honey, and if these changes may be utilised to authenticate Rhododendron honey. Before conducting volatile analysis, certain physicochemical properties were examined to eliminate samples that were inaccurately labelled.

When comparing the free acidity of Austrian Rhododendron honey to Rhododendron honey from Turkey (i.e., honey from mainly *R. ponticum*) (Akgün et al., 2021) it was found that the Austrian honey had lower levels (23.3 mEq/kg) compared to the Turkish honey (34.33 mEq/kg). Additionally, the Austrian honey had a higher electrical conductivity (381 μS/cm) compared to the Turkish honey (320 μS/cm).

Volatile profiles

Rhododendron honeys had a distinct profile of volatile compounds compared to all other types, with the most significant differences in the content of limonene/β-phellandrene, linalool, lilac aldehyde stereoisomers A and B, *p*-cymene, pinocarvone,

Table 3. Cross-validated predictions based on a model calculated from Rhododendron, flower and honeydew honeys. (accuracy: 0.90, no information rate [NIR]: 0.35, *p*-value [Acc > NIR]: 3.22^{-10}).

Label	Flower honey [%]	Honeydew honey [%]	Rhododendron honey [%]
Rhododendron honeys	0	0	100
	0	0	100
	0	0	100
	0	0	100
	100	0	0
	0	0	100
	0	0	100
	0	43	57
	0	60	40
Flower honeys	100	0	0
	6	0	94
	100	0	0
	100	0	0
	100	0	0
	100	0	0
	100	0	0
	100	0	0
	100	0	0
	100	0	0
	100	0	0
Honeydew honeys	0	100	0
	0	100	0
	0	100	0
	0	100	0
	0	100	0
	0	100	0
	0	100	0
	0	100	0
	0	100	0
	0	100	0
	0	100	0
Mountain honeys	*87*	*13*	*0*
	49	*51*	*0*
	0	*0*	*100*
	0	*91*	*9*
	0	*100*	*0*
	1	*0*	*99*
	99	*1*	*0*
	0	*100*	*0*
Mixed flowers/honeydew honeys	*100*	*0*	*0*
	25	*75*	*0*
	2	*98*	*0*
	91	*9*	*0*
	89	*11*	*0*
	0	*100*	*0*
	2	*98*	*0*

Each line represents a sample; the percentages indicate the probability calculated after leaving this sample out of LDA calculation. "Mountain honeys" and "flower/honeydew mix" (in italics) were treated as unknown samples and predicted to the three classes of the model.

trans-3-caren-2-ol, and the fruity ketone 1,4-dimethyl-δ-3-tetrahydroacetophenone.

Limonene/β-phellandrene, linalool and *p*-cymene are monoterpenes frequently occurring in plants and are commonly found in the volatile compounds of *Rhododendron* species (Innocenti et al., 2010; Judžentiene et al., 2012; Qian et al., 2019), often as predominant compounds. However, it was observed that certain *Rhododendron* species lack these compounds (Tasdemir et al., 2003). Regrettably, there is currently insufficient information available regarding the volatile compounds produced by the Rhododendron species that contribute to the Alpine Rhododendron species. 1,4-dimethyl-δ-3-tetrahydroacetophenone likely shares the same precursor as 2-aminoacetophenone and 3-aminoacetophenone, which is indole-3-acetic acid (Simat et al., 2004). The compound 2-aminoacetophenone was suggested as an indicator for Rhododendron honeys by Senyuva et al. (2009), but it was not detected in Alpine Rhododendron honey (Bonometti et al., 2022).

The lilac aldehydes are formed from linalool, as demonstrated *in-vitro* by Burkhardt and Mosandl (2003). They fed *Syringa vulgaris* with ^{18}C-labeled linalool and identified lilac aldehydes amongst the transformation products. These aldehydes occur in numerous plants (Dötterl et al., 2006) and can also be found in some types of honey (Jerković et al., 2009; Senyuva et al.,2009). They have been identified as effective biomarkers for citrus honey (Escriche et al., 2011; Karabagias et al., 2017; Escriche et al., 2023), with the additional advantage of a remarkable stability during prolonged storage (Da Silva et al., 2020). The Rhododendron honeys from Piedmont (Bonometti et al., 2022) included lilac aldehydes, which were also found in the Rhododendron honeys

studied here, originating from the same Rhododendron species. However, Bonometti et al. (2022) suggested that β-damascenone rather than lilac aldehydes, should be considered as biomarkers. The concentration of β-damascenone in our study was rather low and did not show any significant differences between the different varieties of honey. Lilac aldehydes were suggested as biomarkers for Turkish Rhododendron honeys (Senyuva et al., 2009), and were detected in the essential oil of one of the source plants (*R. ponticum*) (Gokturk et al., 2023). Since the lilac aldehydes are present in some species' honeys, these compounds are not specific but non-specific biomarkers (Jerković & Kuś, 2014). Although non-specific honey biomarkers do not directly address the question of origin, their limitation to only a few honeys results in a valuable puzzle element in honey source identification.

Honeydew honeys were significantly different in two compounds, dehydrosabinene and *cis*-linalool oxide. Dehydrosabinene is a rare compound discovered in the wood oil of *Pinus sylvestris* (Wang et al., 2022) and other pine species (Hajdari et al., 2015). The prevalence of conifer aphids from the Lachnidae family in Austrian honeydew (Pechhacker, 1988) may account for the contrasting characteristics compared to Piedmontese honeydew, which is believed to be influenced by the presence of *Metcalfa pruinosa* insects living on *Salix* species (Bonometti et al., 2022).

In contrast to elevated dehydrosabinene levels, the concentration of *cis*-linalool oxide was relatively low at 0.9%, while flower honey showed a concentration of 4.1% and flower honeys of 3.2% (Table 2). Linalool and its derivatives, such as *cis*-linalool oxide, are typical floral volatile compounds that may account for their near absence in honeydew honeys. Italian honeydew honeys (Bonometti et al., 2022) and Polish honeydew honeys (Kuś et al., 2017), however, were shown to have significant levels of *cis*-linalool oxide, which makes it unsuitable as a marker compound.

Methyl salicylate, a compound often found in honeydew honeys (Bonometti et al., 2022; La Fuente et al., 2007; Tananaki et al., 2007) was absent in the honeydew honeys we analysed. La Fuente et al (2007) proposed that *Salix* spp. are the source plants responsible for the presence of methyl salicylate in honeydew honeys. Given the prevalence of Pinaceae as the primary source of Alpine honeydew honeys, it is not unexpected that methyl salicylate is not present.

Authentication of rhododendron honey by discriminant analysis

The discrimination results obtained in this study have shown considerable promise. In the study of Bonometti et al. (2022), a distinct statistical methodology using principal component analysis and k-nearest neighbours was employed. The results revealed that a mere 25% of Rhododendron honeys were accurately categorised. Half of the Rhododendron honeys in the study of Bonometti et al. (2022) were classified as acacia or lime tree, two species that were not examined in our research.

An effective categorization relies on significant differences between groups and the reference set. In our situation, we did not solely depend on labels, but instead utilised certain physicochemical criteria to eliminate samples that were clearly mislabelled. Nevertheless, the reference set had imperfections, as evidenced by the classifications of the cross-validated sample. Integrating pollen analysis might have served as an extra criterion to enhance the accuracy of the model. Some of the samples could have been accurately labelled as Rhododendron, although identifying them as flower or mountain honeys was equally appropriate. Nevertheless, it represented a lost chance for beekeepers.

Conclusions

Certain honey varieties might be regarded as honey specialities that differentiate the product line and support higher market prices since they are exclusive to a particular geographic area. More stringent quality control is required for honey specialities to increase consumers' confidence in these elusive goods. One such honey speciality is Alpine Rhododendron honey. Despite being distinct from the other honeys, this honey did not contain any unique volatile compounds. However, Alpine Rhododendron honey's volatile chemical fingerprint was shown to be different from both, honeydew and flower honeys, allowing a clear distinction from other honeys of the same area.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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